

Branching out to gain control: how the pre-TCR is linked to multiple functions

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Development of T cells with an $\alpha\beta$ T-cell receptor (TCR) is regulated with great precision by what could be regarded as a developmental forerunner of the TCR, the so-called pre-TCR. This receptor triggers signals for survival, expansion and differentiation in pre-T cells, but the molecular events controlled by the pre-TCR remain ill-defined. How are the pre-TCR signals that lead to survival distinguished from those that lead to proliferation or inhibition of rearrangements at the TCR β locus? We argue that pre-T cells that have received survival signals from the pre-TCR do not unfold a pre-existing imprinted program of proliferation and differentiation. Rather, pre-TCR-driven signals play an instructive role in promoting development of double-positive (DP) thymocytes, and the pre-TCR signaling pathway required for cessation of TCR β chain rearrangements is distinct from that which regulates other aspects of $\alpha\beta$ T-cell development. Pre-TCR-driven selection of $\alpha\beta$ T-cell fate thus emerges as a multi-branched process in which the different cell-fate decisions are orchestrated by different signaling pathways.

How is signaling specificity achieved by the pre-TCR during selection of T-cell fate? Like the TCR, this receptor controls many functions, and recent studies define which pathways couple the pre-TCR to the molecular events controlling survival, proliferation, allelic exclusion at the TCR β locus, and further differentiation.

polypeptides, referred to as CD3 proteins. Cells that have undergone β selection progress to the CD4⁺CD8⁺ double-positive (DP) stage (reviewed in Refs 1, 3) via CD8⁺ immature single-positive (ISP) intermediates. Meanwhile, pre-TCR signals terminate rearrangements at the TCR β locus⁸, resulting in allelic exclusion, and TCR α gene transcription and rearrangements are initiated. Although pre-TCR signals might not be absolutely required for TCR β rearrangements to occur⁹, pre-TCR signals are necessary for efficient induction of TCR α gene transcrip-

tion during the DN to DP transition¹⁰⁻¹². Following completion of TCR α rearrangements and production of the clonotypic TCR $\alpha\beta$ -CD3 complex, DP thymocytes are subjected to a rigorous positive and negative selection process (reviewed in Ref. 5) that forms the repertoire of CD4⁺ and CD8⁺ single positive (SP) cells. We shall summarize below the composition and signaling properties of the pre-TCR, and address the different functions it controls in $\alpha\beta$ T-cell development. Its instructive role in $\alpha\beta$ versus $\gamma\delta$ T-cell development was recently addressed elsewhere¹³, and will be omitted here owing to space constraints.

Pre-TCR driven selection of $\alpha\beta$ T-cell fate

Developing thymocytes follow a strictly regulated developmental program that is initiated after fetal liver or bone-marrow-derived precursor cells enter the thymus. Transition from earlier to later stages depends on signals provided by cytokine receptors (reviewed in Refs 1, 2), as well as by the pre-TCR (reviewed in Refs 1, 3) and the TCR (reviewed in Refs 4, 5). The most immature thymocytes reside within the CD4⁺CD8⁻ double-negative (DN) population (Fig. 1), where TCR gene rearrangements are initiated once CD25 is expressed, and commitment to the T-cell lineage becomes irreversible. TCR β gene rearrangements are completed at the CD44⁺CD25⁺ stage.

This latter event represents perhaps the most crucial cell-fate-determining event in the development of $\alpha\beta$ T cells: only cells that generate a functional TCR β chain mature from the CD44⁺CD25⁺ to the CD44⁺CD25⁻ stage and are selected to develop into $\alpha\beta$ T cells^{6,7}. This process has been termed 'β selection' and is controlled by the pre-TCR (Ref. 3), a multi-subunit receptor complex comprising the TCR β chain, a pT α chain, and several non-covalently associated

Subunit requirements for pre-TCR expression and function

Pre-TCR complexes have a subunit composition¹⁴ that is comparable to $\alpha\beta$ TCR complexes (Fig. 2). They contain a TCR β chain associated with the invariant CD3 γ , δ , ϵ and ζ chains, but the TCR α chain has been replaced by an invariant 33 kDa glycoprotein subunit termed pre-T α (pT α)^{15,16}. pT α has only one immunoglobulin (Ig)-like exodomain loop, compared with two in the TCR α and β chains, prompting speculation that pre-TCRs might contain an additional subunit 'V-preT' (by analogy with the 'V-preB' present in the pre-B cell) that pairs with the V-region of β . Assembly of nascent pre-TCR complexes has not been studied in detail, but the subunit composition of the pre-TCR suggests that its assembly should be similar to that of the $\alpha\beta$ TCR, implying that all known subunits are needed. However, unlike the $\alpha\beta$ TCR, which requires all of its components ($\alpha\beta\gamma\delta\epsilon\zeta$) for optimal surface expression and function, gene-targeting experiments¹⁷⁻²⁶ have revealed that pre-TCR expression (and function)

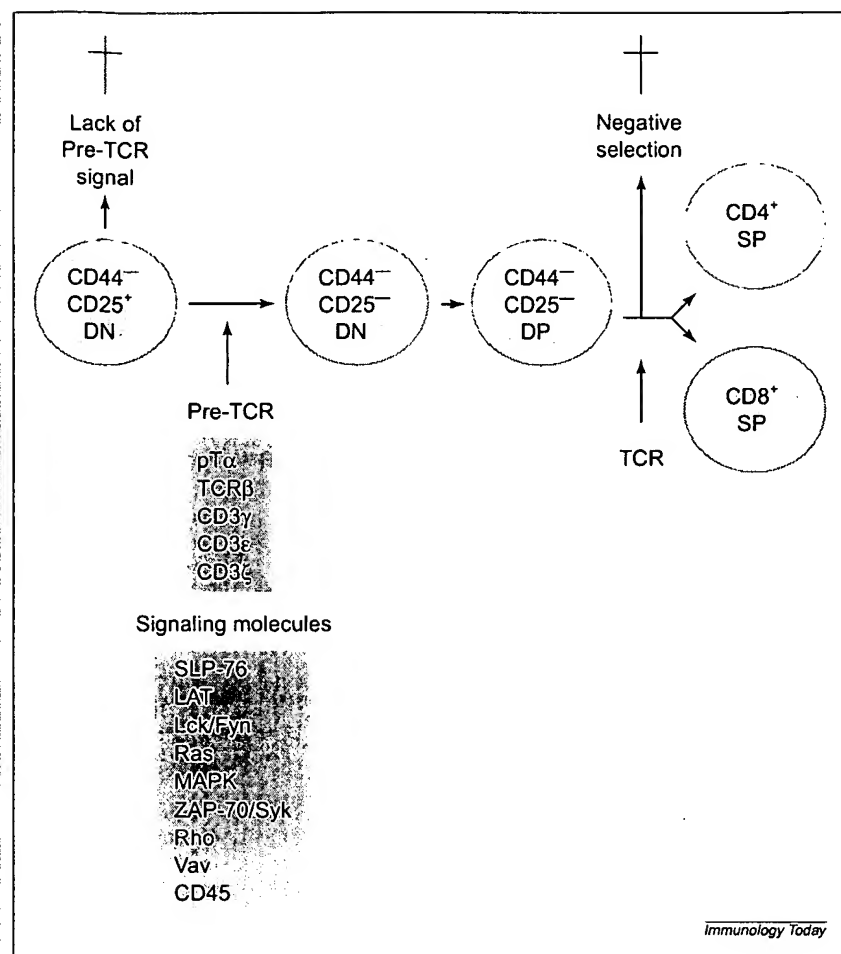


Fig. 1. Schematic view of the major selection steps in $\alpha\beta$ T-cell development. The pre-TCR controls transition from the CD44⁺CD25⁺ to the CD44⁺CD25⁺ stage in the CD4⁺CD8⁺ (double-negative, DN) compartment. At the CD4⁺CD8⁺ (double-positive, DP) stage, TCR signals result either in CD4⁺ or CD8⁺ single-positive (SP) lineage fate, or in cell death (negative selection). The pre-TCR subunits and signaling molecules required for pre-TCR expression and function are depicted in boxes. When the pre-TCR is absent or dysfunctional, DN cells die. Abbreviations: LAT, linker for activation of T cells; TCR, T-cell receptor.

is less affected by ablation of some of its biochemically defined subunits than $\alpha\beta$ TCR expression (see Table 1). Most notably, CD3 ζ deficiency only partially impairs pre-TCR function, and CD3 δ deficiency has no effect at all. Because pre-TCRs are distinguished from $\alpha\beta$ TCRs only by the substitution of pT α for TCR α , these peculiarities of pre-TCR expression are presumably caused by pT α .

Following TCR β gene rearrangement at the CD44⁺CD25⁺ stage, very low levels of the pre-TCR are expressed on the surface. The surface expression of pre-TCR complexes appears to be low because of two structural features of pT α that limit the extent of pre-TCR assembly: the lack of a V-region domain and lack of its connecting piece (CP), which supports only very weak association with CD3 ζ subunits²⁷. Consequently, most pT α in DN thymocyte cell lines (and presumably normal DN cells) remains in the endoplasmic reticulum²⁸.

Initiation of pre-TCR signaling

Although most lymphocyte surface receptor complexes are activated by ligand engagement, for which antibody (Ab) stimulation frequently serves as an effective surrogate, this does not appear to be true for the pre-TCR complex. In fact, Ab engagement of the pre-TCR *in vivo* arrests maturation of thymocytes prior to the DP stage²⁹. Moreover, the potential ligand-binding exodomains of pT α and TCR β are dispensable for pre-TCR function^{30,31}. These data suggest that engagement by a specific ligand is not responsible for initiation of pre-TCR signaling *in vivo*. However, it is likely that the pre-TCR needs to be transported to the cell surface of immature thymocytes³², presumably to meet crucial signaling components.

How then is signaling initiated? It appears that the pre-TCR has a unique capability to transduce β -selection signals even when expressed at low levels and when not engaged by a surface ligand. There are several hypotheses to explain this phenomenon, all of which have in common the concept that pre-TCRs are constitutively active signaling complexes, and that this constitutive signaling ability is conferred upon the pre-TCR by some unique property of pT α (Refs 30, 32). If so, then that 'property' of pT α probably maps to its CP or its transmembrane domain, because the Ig loop and cytoplasmic tail are dispensable for function, at least when overexpressed³³. It is unclear how pT α might confer constitutive signaling ability upon the pre-TCR, but it has been suggested that pT α does so by directly targeting pre-TCR complexes into lipid microdomains, which are enriched in glycolipids and signaling molecules involved in T-cell activation (also known as rafts, GEMs, DIMs) (reviewed in Ref. 34).

In agreement with this suggestion, a recent report³⁵ suggests that pT α is palmitoylated, and that its palmitoyl moiety targets the pre-TCR to lipid microdomains. Further investigation is required to resolve whether this is indeed the basis for ligand-independent signaling of the pre-TCR.

Proteins involved in pre-TCR signaling

Regardless of how pre-TCR signaling is initiated, its CD3 components endow it with its molecular sensor properties. Insights into the signaling components involved have been obtained largely through natural or engineered mutations, and through functional inactivation studies in fetal thymus organ cultures (Tables 1, 2).

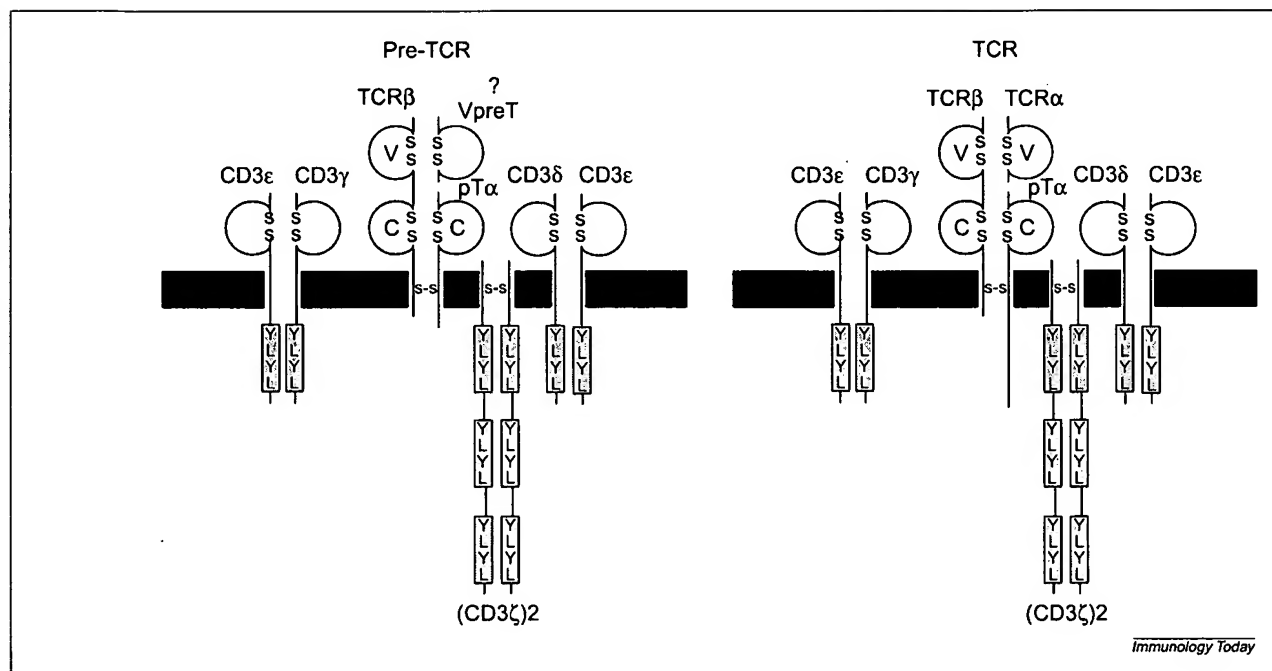


Fig. 2. Subunit composition of the pre-TCR and the TCR. Pre-TCRs are distinguished from $\alpha\beta$ TCRs only by the substitution of $pT\alpha$ for $TCR\alpha$. The $pT\alpha$ chain has only one immunoglobulin-like exodomain loop, and the $VpreT$ -loop depicted here is based solely on speculation: by analogy with the $VpreB$ -loop in the pre-BCR, such a component might exist. Another difference between the pre-TCR and the TCR is that $CD3\delta$ is difficult to detect and not required for pre-TCR function, whereas in the $\alpha\beta$ TCR, it is required for both assembly and function. The 'YLYL' motifs shared by all CD3 chains represent the immunoreceptor tyrosine-based activation motifs (ITAMs) that, after phosphorylation, couple the pre-TCR and the TCR to the adaptors and kinases critical for signaling. Abbreviation: TCR, T-cell receptor.

The CD3 components that form part of the pre-TCR (and of the mature TCR, for that matter) contain one or several immunoreceptor tyrosine-based activation motifs (ITAMs) within their cytoplasmic domain. As in the TCR, these ITAMs are phosphorylated by activated Src-family protein tyrosine kinases (PTKs) which, together with their regulatory enzymes, play critical roles in pre-TCR signaling^{33,36-43}. Phosphorylation of the ITAMs creates docking sites for SH2-domain-containing Syk-family PTKs, ZAP-70 and Syk, both of which contribute to pre-TCR function^{44,45}.

More distal events in pre-TCR signaling are regulated by adaptors, exchange factors and GTPases, all of which are involved in mature TCR signaling. Indeed, the adaptor proteins SLP-76 and LAT (linker for activation of T cells) are crucial for pre-TCR signaling⁴⁶⁻⁴⁸, as are the GTPases p21ras (Ref. 49) and Rho (Refs 50, 51), and (to a lesser extent) the guanine nucleotide exchange factor Vav (Refs 52-55). In addition, the Ras-Raf-MAPK-ERK cascade has been implicated in pre-TCR signaling, in that introduction of constitutively active components of this pathway restores development of pre-TCR deficient thymocytes to the DP stage^{56,57}. Finally, a novel reporter plasmid system for detecting ERK activation within the developing thymocytes showed that formation of the pre-TCR leads to activation of this serine/threonine kinase⁵⁸. ERK's substrate, the transcription factor Elk-1, can also be activated by the JNK pathway, but inactivation of key components in this pathway does not affect β selection⁵⁹⁻⁶¹.

How might Elk-1 and other transcription factors regulate β selection? This is still an area of much speculation because the nuclear targets of the signaling pathways triggered by the pre-TCR are largely unknown. Nevertheless, several transcription factors have been implicated in β selection (Table 2), although their putative targets at this developmental stage remain to be defined. The E family of bHLH (basic helix-loop-helix) transcription factors (E2-2, HEB and E2A) plays key roles in multiple developmental pathways, and affects the DN to DP transition: mice lacking HEB (Ref. 62) have a decrease in DP and increase in ISP thymocytes. The Ikaros family of hematopoietic-specific transcription factors are essential for T-cell lineage commitment, and act beyond the DN stage: pre-T cells lacking Ikaros can perform β selection without the pre-TCR (Ref. 63). Ablation of both the high mobility group (HMG) box transcription factors Tcf-1 and Lef-1 prevents thymocyte development beyond the ISP stage¹⁰. The early growth response gene family member 1 (Egr-1) is upregulated in β -selected cells, and its enforced expression relieves the developmental arrest in pre-TCR-deficient thymocytes⁶⁴. Egr family members regulate expression of many gene targets of β -selection signals, including $TCR\alpha$, $pT\alpha$ and the Rag genes (M. Carleton *et al.*, unpublished). Investigations are currently under way to discover which other aspects of β selection are controlled by this family of transcription factors.

In conclusion, many of the membrane-proximal signaling effectors activated by the mature TCR are used in pre-TCR signaling. How

Table 1. Mutations that have provided insights in the regulation of $\alpha\beta$ T-cell lineage differentiation^a

Deficiency	Phenotype	Refs
pT α	Block at CD44 ⁺ CD25 ⁺ stage Rescue with activated Lck, tailless pT α anti-CD3	8, 17
TCR β	Block at CD44 ⁺ CD25 ⁺ stage Rescue with anti-CD3	25 75
TCR α	Block at DP stage	25
CD3 γ	Block at CD44 ⁺ CD25 ⁺ stage Rescue with activated Lck, anti-CD3, loss of p53	20 38
CD3 δ	Block at DP stage	14, 18
CD3 ϵ	Block at CD44 ⁺ CD25 ⁺ stage	19, 26
CD3 ζ	Partial block at DN to DP transition	21-24
Lck	Partial block at DN to DP transition Partial rescue (Rag ^{-/-} \times Lck ^{-/-}) with anti-CD3	36 76
Fyn	No β selection defect	39, 40
Lck/Fyn	Severe block at CD44 ⁺ CD25 ⁺ stage	39, 40
Csk	Bypasses need for pre-TCR and TCR	41
CD45	Partial block at DN to DP transition	42, 43
ZAP-70/Syk	Block at CD44 ⁺ CD25 ⁺	44, 45
SLP-76	Block at CD44 ⁺ CD25 ⁺ No rescue with anti-CD3	46, 48
LAT	Block at CD44 ⁺ CD25 ⁺	47
Vav	Partial defect in DN to DP transition	52-55
Rho	Block at DN to DP transition	50, 51
Rag	Block at CD44 ⁺ CD25 ⁺ stage Rescue with activated Lck, with activated Ras, Raf, with DN-FADD, anti-CD3	77, 78 37 49, 56, 57 69 75, 79, 80

^aAbbreviations: TCR, T-cell receptor; DP, double-positive (CD4⁺CD8⁺); DN, double-negative (CD4⁻CD8⁻); DN-FADD, dominant-negative Fas-associated death domain; LAT, linker for activation of T cells.

then are these shared signaling events translated into distinct biological functions? Whereas both the mature TCR and the pre-TCR can trigger anti-apoptotic events and proliferative signals, the pre-TCR triggers several additional outcomes, including termination of TCR β locus rearrangements and induction of CD4 and CD8 expression. Are these distinct cellular outcomes the consequence of a common signaling machinery defined by different cellular contexts? For other pathways, notably Ca²⁺ signaling⁶⁵, this certainly can be the case. The specificity of Ca²⁺ signaling is determined in part by selective use of downstream transcriptional targets that are differentially expressed in certain cell types. Furthermore, differences in concentrations of

crucial cytoplasmic and nuclear mediators between cell types might dictate the outcome of generic signals. Determining to what extent the differences in outcome between pre-TCR and TCR signaling might be shaped by cellular context remains a task for the future. Possibly, some answers will be obtained in the not too distant future, through representational difference analysis and gene expression arrays. Moreover, the pre-TCR might produce different biological outcomes by coupling to cytoplasmic mediators distinct from those used by the TCR; identification of such mediators might be a fruitful area of research.

How to traverse the β selection checkpoint with one sensor

Why must the signaling network used by the pre-TCR be so elaborate? The answer probably lies in the many different functions it controls. First, DN cells lacking a pre-TCR have a survival defect, implicating the pre-TCR in providing crucial anti-apoptotic signals. Second, transition from the DN to the DP stage is accompanied by prolific expansion, and signaling through the pre-TCR is essential for entering this burst of cell proliferation. Third, once functional rearrangements of one TCR β allele have occurred and the pre-TCR is formed, pre-TCR signals terminate rearrangements at the remaining TCR β allele, thus ensuring allelic exclusion. Finally, pre-TCR signals control further maturation to the DP stage, regulating numerous genes, including those required for induction of CD4 and CD8 expression, for downmodulation of pT α gene expression, and those involved in transactivation of the TCR α locus prior to its rearrangement. Here, we consider how signal specification can be achieved by the pre-TCR (Fig. 3), searching for clues in gene ablation and gene complementation studies.

The notion that rescue from cell death is not the only task of the pre-TCR has become clear as the molecular mediators involved in its pro-survival role are explored. The survival defect of pre-TCR-deficient CD25⁺ DN cells, or CD25⁺ DN cells whose pre-TCR signaling has been attenuated (due to loss of Rho function) is manifested only in the presence of the tumor suppressor gene p53 (Refs 66, 67). Importantly, expression of transgenic Bcl2 does not rescue survival and differentiation of CD3 γ -deficient mice³⁸, consistent with the earlier report⁶⁸ that overexpression of Bcl2 can rescue the survival defect in interleukin-7-receptor-deficient mice, but not that of Rag1-deficient thymocytes lacking the pre-TCR.

Table 2. Transcriptional control of the DN to DP transition*

Transcription factors implicated in β selection	Observation	Refs
bHLH (E family)	HEB deficiency results in an increase in ISPs and decrease in DP	62
Ikaros family	Without Ikaros function, transition to the DP stage becomes pre-TCR-independent	63
HMG family	Mice lacking both Lef-1 and TCF-1 fail to transit beyond the ISP stage	10
Egr family	Enforced Egr-1 expression allows transition to the ISP stage in Rag-deficient mice	64

*Abbreviations: bHLH, basic helix-loop-helix; DN, double-negative ($CD4^-CD8^-$); DP, double-positive ($CD4^+CD8^+$); Egr-1, early growth response gene family member 1; HMG, high mobility group; ISP, immature single-positive ($CD8^+CD3$); TCR, T-cell receptor.

The studies in p53-deficient mice are important not only because they place inactivation of p53 directly downstream from pre-TCR signaling, but also because they reveal what p53-deficiency cannot achieve. Genetic removal of p53, in isolation, fails to rescue thymic cellularity and only minimally rescues differentiation of the pre-TCR-deficient thymocytes to the DP stage⁶⁶. Moreover, in mice with a crippled pre-TCR, p53-deficiency restores expansion and differentiation to the DP stage only after a protracted time period, despite full rescue of pre-T-cell survival³⁸. Defective pre-TCR driven proliferation and differentiation signals are the most likely explanations for these findings, underscoring the notion that the signal for survival bifurcates upstream from those regulating proliferation and further maturation. In addition, the signals for maturation and proliferation might be separable, because expression of dominant-negative Fas-associated death domain (DN-FADD) in pre-T cells that lack a pre-TCR rescues survival and differentiation but not proliferation⁶⁹. In agreement with these findings, pre-T cells that express DN-FADD but lack a pre-TCR are resistant to the proliferative response that can be induced by CD3 ligation⁶⁹. These findings suggest not only that inactivation of FADD can obviate the need for pre-TCR survival signals, but also that FADD is required for pre-TCR-driven proliferation signals and not for maturation signals.

Which other signaling effectors might be positioned at key branch points? As mentioned above, activation of the Src-family PTK Lck appears necessary and sufficient to provide all pre-TCR driven β -selection events, because expression of an activated Lck transgene in numerous mouse strains with natural or engineered pre-TCR defects (Table 1) can fully restore the differentiation program normally orchestrated by the pre-TCR. However, Lck's ability to trigger proliferation and differentiation can be distinguished from its ability to drive allelic exclusion by its dependence on Rho (Ref. 70), suggesting a branching model for pre-TCR signaling downstream of Lck. All β -selection events, including allelic exclusion, are dependent on SLP-76 (Refs 46, 48, 71), probably due to the fact that SLP-76 is a critical scaffold protein for several adaptors, regulators of Ras signaling, and phospholipase C γ 1 (PLC γ 1)^{72,73}. Further important clues about bifurcation of pre-TCR signals

were provided last year, when two groups reported on the role of the Ras-Raf-MAPK pathway in β selection^{56,57}. In the absence of a pre-TCR, active Ras-V12 (Ref. 57) and active Raf-CAAX (Ref. 56) expressed in Rag-deficient thymocytes trigger survival and proliferation, and transition to the DP stage. However, in normal mice, they

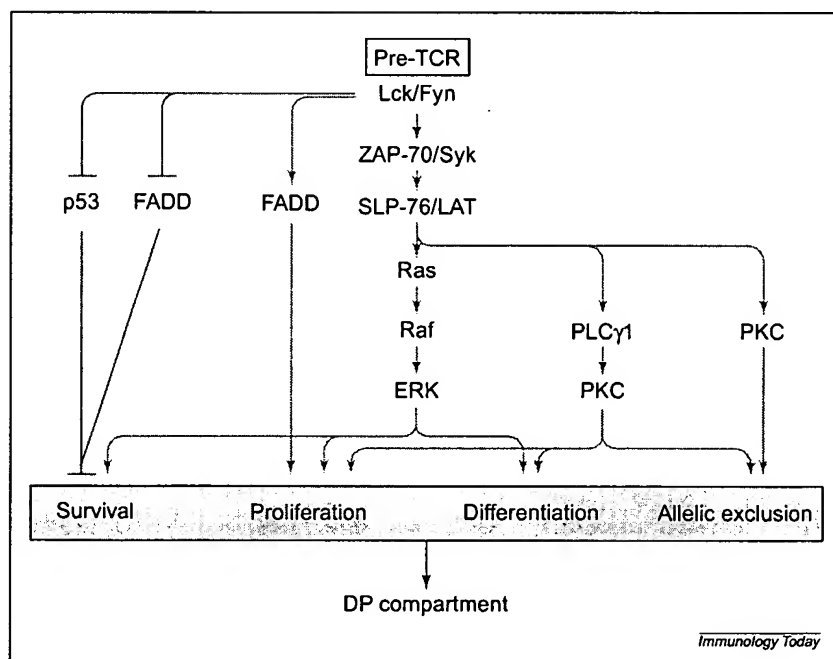


Fig. 3. Proposed model for signal specification by the pre-TCR. Gene ablation and gene complementation studies have implicated the signaling mediators depicted here. Other components involved in signal specification are likely to be defined, but the present-day knowledge of the mediators required for pre-TCR driven selection already support this multi-branched, instructive model. Activation of Lck is necessary and sufficient for all pre-TCR-driven selection events. Signals for survival bifurcate upstream of those for proliferation and differentiation, and the Ras-Raf-ERK pathway regulates survival, proliferation and differentiation. By contrast, signals for TCR β allelic exclusion bifurcate upstream of Ras and downstream of SLP-76/LAT, and require PKC activation. Abbreviations: DP, double-positive ($CD4^+CD8^+$); FADD, Fas-associated death domain; LAT, linker for activation of T cells; PLC γ 1, phospholipase C γ 1; PKC, protein kinase C.

fail to induce termination of TCR β chain rearrangements. These studies indicate that TCR β allelic exclusion is not a by-product of differentiation, but actually requires specific signals that bifurcate upstream of the Ras-Raf-MAPK cascade. Together with the above cited role of Lck and SLP-76 in allelic exclusion, these findings place the bifurcation of signals for allelic exclusion upstream of Ras activation, and downstream of Lck/ZAP-70-mediated phosphorylation of SLP-76.

Because one of the roles of SLP-76 is triggering protein kinase C (PKC) activation by PLC γ 1, PKC appears to be another likely point of signal bifurcation. The PKC- θ isoform has been implicated in TCR signaling in mature T cells, but does not have a specific role in β selection: mice lacking PKC- θ have a severe defect in mature T-cell activation, but normal thymocyte development⁷⁴. Might other isoforms of PKC then be involved? Indeed, that does appear to be the case: PKC is activated upon formation of the pre-TCR complex (A.M. Michie *et al.*, unpublished), and this activation results in proliferation and maturation of DN thymocytes. Moreover, an activated form of PKC- α was shown to enforce allelic exclusion at the TCR β locus (A.M. Michie *et al.*, unpublished). PKC signals are thus critical mediators of pre-TCR signal specification, and can be positioned at the branch point where signals leading to proliferation and differentiation diverge from those enforcing allelic exclusion. These findings illustrate that the pre-TCR and TCR can couple to different PKC isoforms. Thus, although the pre-TCR and TCR share many proximal signaling events, the different biological outcomes of triggering these receptors might relate in part to their coupling to different mediators. Identification of additional pre-TCR-binding mediators will be critical in understanding its specific biological role, as will be the identification of specific target genes.

Concluding remarks

One of the most fascinating challenges in T-cell biology is to define how signaling specificity is directed by TCRs and pre-TCRs during selection of T-cell fate, be it in mature T cells or in their precursors. From data such as those reviewed above, we arrive at the view that the pre-TCR-driven transition from the DN to the DP compartment is a multi-branched program, in which the pathways leading to developmental progression and expansion can be distinguished from those resulting in survival and termination of TCR β chain rearrangements. The inevitable conclusion is that the pre-TCR couples to multiple functions through different pathways, in ways yet to be fully defined. Of course, this situation is no different than that of the mature TCR, whose differential signaling capabilities have been studied for many years. Because both receptors are associated with an elaborate signaling network, subtle distinctions in initial activation or recruitment of proximal signaling effectors can be propagated into different signaling outcomes, controlling specific cell fate decisions and differentiation steps.

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Letters

Do glucocorticoids participate in thymocyte development?

There is a substantial body of evidence suggesting that glucocorticoids affect T-cell development. In this issue, Godfrey and colleagues¹ review this literature in light of their recent finding that prenatal thymocyte development is grossly normal in glucocorticoid receptor knockout (GR^{-/-}) mice.

Some of the data in this area have been generated either in manipulated normal animals or in transgenic mice that express antisense GR transcripts in many tissues. Because of feedback regulation of systemic glucocorticoids on the hypothalamus–pituitary–adrenal (HPA) axis, and the fact that glucocorticoids are also produced locally in the thymus, it is difficult to know in these studies exactly how glucocorticoids or weak agonists/antagonists like RU-486 exert their effects. We have attempted to minimize the influence of adrenal steroids and the HPA axis by using two different experimental approaches: expression of GR antisense transcripts under the control of the *lck* proximal promoter – that is, only in thymocytes (TKO

mice) – and fetal thymic organ culture (FTOC) in which glucocorticoid biosynthesis is inhibited. Both approaches have provided consistent data suggesting not only a role for glucocorticoids in T-cell development, but also possible molecular mechanisms. Do the data with the GR knockout mice now preclude a role for glucocorticoids in thymocyte development? We believe that at this time we do not have sufficient information to reach a conclusion.

We have proposed that glucocorticoids, by interfering with the nuclear consequences of TCR-mediated activation, set the signaling thresholds for different selection outcomes. This is supported by the finding that CD5 expression on double positive (DP) thymocytes, a sensitive indicator of TCR occupancy, is upregulated in an MHC-dependent manner when glucocorticoid signaling is attenuated *in vivo* or *in vitro*². Antigen-specific selection has been addressed in several ways. Addition of metyrapone, an inhibitor of corticosterone synthesis, to FTOC caused the apoptosis of antigen-specific TCR transgenic DP thymocytes that normally undergo positive selection (reversed by corticosterone), but had no effect on viability if the MHC haplotype was incapable of presenting the selecting antigen³. In another approach, the number of T cells expressing TCRs that

are positively selected (identified by the use of particular V β regions) was reduced by introduction of the GR antisense transgene⁴. Even more strikingly, TKO mice were found to have an altered TCR repertoire, being nonresponsive to pigeon cytochrome c but responding normally to other complex antigens⁵. Is selection altered in the GR^{-/-} mice? Based on the available data it is not possible to say. Negative selection in response to potent stimuli appears to be grossly normal, but without dose–response curves it is not possible to say whether GR^{-/-} DP thymocytes are unusually sensitive. Positive selection is even more problematic. Positive selection clearly occurs in GR^{-/-} mice, but does the TCR repertoire differ from that of wild-type animals? At this time we do not know, but characterization of the effect of GR loss on MHC-dependent V β use in normal animals, and selection in antigen-specific $\alpha\beta$ TCR transgenic mice, should shed light on this issue.

The major documented difference between thymuses from GR^{-/-} and TKO mice or from normal mice cultured in FTOC with metyrapone is that only the former have normal numbers of DP cells. Is it possible to reconcile these very different findings? One possibility is that there might be strain-specific differences between the mice. We